# Grasses and the resource availability hypothesis: the importance of silica-based defences

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# Grasses and the resource availability hypothesis: the importance of silica-based defences

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Running title: Grass defence strategies and palatability to voles

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**Summary**

1. The resource availability hypothesis (RAH) predicts that allocation of resources to antiherbivore defences differs between species according to their growth rate. As a test of the RAH, we assessed the growth and defence investment strategies of 18 grass species and compared them to vole feeding preferences. In addition, we assessed the effectiveness of silica, the primary defence in many grasses, in influencing vole feeding behaviour.
2. Across species, we found that there was a strong negative relationship between the overall investment in defence and growth rate, thus supporting predictions of the RAH. However, no such relationship was found when assessing different antiherbivore defences individually, suggesting significant variation in the investment in specific defence strategies (namely phenolic concentration, silica concentration and leaf toughness) of different grass species.
3. Silica was the most influential defensive factor in determining vole feeding preference. Increasing leaf silica concentration experimentally deterred vole feeding in three of the five species tested, and was sufficient to alter feeding preference ranks between species. The strong positive relationship between silica concentration and leaf abrasiveness, both when assessed within and between species, suggests that increased abrasiveness is the mechanism by which silica deters feeding.
4. Although often considered tolerant of herbivore damage rather then defended against it, grasses do follow predictions of defence allocation strategy based on their growth rates, and this affects the feeding behaviour of generalist grass feeding herbivores.

Keywords: Feeding preference, grass, growth rate, herbivory, *Microtus agrestis*, resource allocation hypothesis, voles.

## Introduction

A central goal in the study of plant-animal interactions has been to understand the factors controlling investment in plant defences as opposed to growth, and the consequences of these allocation patterns for herbivore behaviour and performance (Herms & Mattson 1982; Hartley & Jones 1993). The resource availability hypothesis (RAH) (Grime 1979; Coley *et al.* 1985) proposes that inherently fast-growing plant species adapted to resource-rich environments have high foliar nutrient concentrations, high tissue turnover rates, low investment in antiherbivore defences and, consequently, high levels of herbivore damage. In contrast, species adapted to resource-poor environments will be slower-growing, with lower tissue nutrient concentration, have greater tissue longevity and higher investment in antiherbivore defences leading to lower levels of herbivory. Replacement of tissue lost to herbivores is relatively more costly for slow growing plant species with long-lived tissues than for fast growing species, whereas diverting resources to defence rather than growth will incur high costs for fast growing species in highly competitive environments.

Although theories of defence allocation, of which the RAH is one (Hartley & Jones 1993), have been the subject of debate, the RAH has gained significant support (Stamp 2003). The RAH and its relationship to herbivore feeding has been extensively tested for woody plant species (Coley 1983; Coley 1985; Matsuki & Koike 2006), but there has been little attention paid to its relevance for growth-defence relationships in grasses. This may reflect the opinion that grasses use re-growth as a strategy to cope with herbivory, rather than large investments in antiherbivore defences (Del-Val & Crawley 2004, 2005). Much of the work into the response of grasses to herbivores has focused on their ability to compensate for defoliation with increased growth rates (McNaughton 1983, 1992). However, grasses do have a combination of physical and structural defences limiting herbivore damage (Vicari & Bazely 1993). Furthermore, recent studies have revealed that silica bodies (phytoliths) deposited in grass leaves act as an effective defence against herbivory (Gali-Muhtsib *et*

*al.* 1992; Massey *et al.* 2006; Massey & Hartley 2006). Phytoliths are thought to increase the abrasiveness of grass leaves, thereby deterring herbivory. They can also reduce the efficiency with which herbivores absorb nitrogen from their food, and thus impact on herbivore growth rates (Massey *et al.* 2006; Massey & Hartley 2006). This key defence in grasses has been relatively neglected in studies of grass-feeding herbivores, but foliar silica concentrations vary dramatically between grass species and can be increased following herbivory (McNaughton & Tarrants 1983; Massey *et al*. 2006; Massey & Hartley 2006). This variation, together with the large range of life history strategies shown by grasses (Grime *et al.* 1988), suggest that theories of growth versus defence investment, such as RAH, may be relevant to this group of plants, but this has not been adequately tested. For silica defences the cost of defence is unclear. Unlike carbon- or nitrogen-based defences, silica is not directly associated with plant growth (Raven 2003). However, recent work has identified an active transport mechanism of silica into the roots of grasses (Ma *et al.* 2006), implying an associated cost.

Feeding by small mammalian herbivores, such as voles, is usually highly selective both within and between plant species (Marquis & Batzli 1989; Hartley *et al.* 1995; Hjältén *et al.* 1996). At times of peak population sizes, voles of the genus *Microtus*, which feed primarily on leaves and shoots of grasses (Ostfeld 1985; Hjältén *et al.* 1996), can affect changes to plant community composition (Moen *et al.* 1993; Howe & Brown 1999; Clay 2001; Howe & Lane 2004). However, there have been relatively few broad scale studies assessing what factors influence vole feeding preferences and previous work has focused on a diverse range of plant species, of which graminoids were only a minor component (Marquis & Batzli 1989; Hjältén *et al.* 1996). To our knowledge, none of the studies comparing interspecific feeding preferences of voles have assessed the impacts of silica on diet choice.

Here we test the factors affecting the palatability of grasses to field voles (*Microtus agrestis* L.) and relate the findings to predictions of the RAH. Specifically we address three key questions:

1. Does the defence strategy of grasses follow the predictions of the resource availability hypothesis? We predicted that slower-growing species, with lower foliar nitrogen concentrations, would have higher levels of antiherbivore defences than faster-growing species.
2. What plant-based factors have the greatest influence on vole feeding behaviour? As with some previous studies, we predicted that growth rates and nutrient concentrations would affect feeding preference, but we also predicted that silica-related abrasiveness would influence vole feeding preference. Furthermore, we predicted that the cumulative defence score (CDS) of a species, as an indication of the allocation to all three primary defences in grasses, would influence vole feeding behaviour.
3. Does manipulation of foliar silica concentration affect vole feeding behaviour? Again, we predict that plants with higher silica concentrations will have higher levels of foliar abrasiveness making them less palatable to voles.

## Material and methods

### GRASS SPECIES AND GROWTH CONDITIONS

We selected 18 commonly occurring European grass species of contrasting palatability and growth rates (Table 1). Grass seeds were germinated on vermiculite until ~2 cm in height before being transplanted into 5×5×5 cm plugs of standard compost mix (10 SHL multipurpose compost: 3 Perlite: 1 sand by volume). Grasses were grown for 12-14 weeks in glasshouses at 15-30 °C with 16:8 light:dark regime.

### PLANT QUALITY MEASUREMENTS

Ten plants of each species were destructively harvested and measurements taken of leaf length and width, as leaf size may offer visual cues for vole feeding. Washed plant samples were oven dried at 60 °C for 48 hours and measurements made of leaf water content, specific leaf area (SLA = leaf dry mass per unit area), growth rates (g dry mass per day), dry mass root:shoot ratio. Dried leaf samples were then ground (in a 'Pulverisette 23', Fritsch, Germany) for subsequent chemical analyses ($n$ = 10 per species for all analyses).

Foliar silica concentration was determined by fusing dried leaf samples (approximately 0.2 g) in sodium hydroxide followed by analysis using the colorimetric silicomolybdate technique (Allen 1989). Total phenolic concentration was assayed using the Folin-Ciocalteau method, with tannic acid standards (Massey *et al.* 2005). Foliar nitrogen and carbon concentrations were analyzed using flash combustion of ground leaf samples (approximately 2.5 mg) followed by gas chromatographic separation (Elemental Combustion System; Costech Instruments) calibrated against a standard of composition $C_{26}H_{26}N_2O_2S$ (Massey *et al.* 2006).

The comparative leaf toughness of fresh leaves was determined by measuring their tensile strength ($n$ = 10) using methods described by Grime *et al.* (1993), a procedure which is valid because both leaf toughness and strength are both closely correlated to the cell wall fraction (Choong 1996). Longitudinal leaf sections of known width were gradually pulled to the point of breakage. The force required to cause breakage was measured from a spring balance, which was then corrected for the width of leaf material. Abrasiveness ($R_z$) of grass samples ($n$ = 10) was determined by grinding a fixed area of fresh grass leaves (5 $cm^2$) against a Perspex plate using a modified Martindale abrasion and pilling tester (Model 404, James H. Heal, Halifax). The degree of scratching on the Perspex was quantified as average scratch depth ($R_z$) using a laser perthometer. The more abrasive

the grass, the rougher the Perspex plate would be after abrasion and hence, the larger the value of $R_z$ (Massey *et al.* 2006).

CALCULATION OF THE CUMULATIVE DEFENCE SCORE (CDS)

As many of the grass species appeared to contain different levels of each of the defence measures, to assess a species overall defence strategy we used combined data on all three primary defences (silica concentration, phenolic concentration and toughness) to generate the CDS of each species. This was calculated as the sum of the relative percentage ranking for each of the major defences. We did not include abrasion in the analysis due to its strong correlation with silica concentration (see results section). For each defence, the 18 grasses were ranked, and the relative investment in each defence was calculated as a percentage of the value for the highest ranking species. The percentage defence level for the three defence types was then summed for each species and this value used as its CDS. This method produces a defence strategy rating relative to all the other species studied, but weighted for the range of values of each defence type. It makes no assumptions about the relative effectiveness of each defence, which would clearly vary with factors such as herbivore identify, but it does allow us to assess the overall defence strategy of each as a single measure and compare this to the species growth strategy and its palatability to voles.

VOLE FEEDING PREFERENCE TRIALS

Voles used in feeding trials were all non-lactating adults from a captive bred colony fed on a standard dried diet (N = 2.97%, C:N = 16.4, $SiO_2$ = 2.64 dry matter: rabbit maintenance diet, B&K Feeds Universal) and fresh leaves of *Triticum avenae*. Voles were limited to water and a dried diet for 24 hours prior to the trials. We conducted ten interspecific feeding choice tests with two intact individuals of each grass species per trial (i.e. 36 plants per trial). The leaf area of each plant was scanned at the start of the trials (AM-200 leaf area meter, ADC). The plants were arranged

randomly in a grid design (6×6 individuals) within a glass tank (45×30×30 cm) containing sawdust in the bottom, level with the base of the shoots of the grasses. One vole was then placed in the cage and left to feed. The stopping rule for trials was when an estimated 50% of total leaf area had been consumed, which took 3-4 hours in trials with 36 plants, after which the remaining leaf area of each plant was measured.

EFFECTS OF FOLIAR SILICA CONCENTRATIONS ON FEEDING PREFERENCE

To assess the impacts of silica on feeding preference we manipulated silica concentrations of five grass species naturally consumed by voles in the field (*Agrostis capillaris*, *Brachypodium pinnatum*, *Festuca ovina*, *Lolium perenne* and *Poa annua*) and conducted interspecific and intraspecific feeding preference trials. Grasses were grown in washed Perlite, an inert growth medium, under glasshouse conditions for 12-14 weeks (15-25 °C, 16/8 h L/D). Plants were transplanted individually into 5×5 cm plugs at approximately 2 cm in height, and watered every 3 days with 25 ml of Hoagland's solution. To half of the plants in the study, 150mg $l^{-1}$ of soluble silica was added as $NaSiO_3.9H_2O$ to the Hoagland's solution (Cid *et al.* 1990). The silica, nitrogen, water, phenolic concentrations as well as the growth rates of grasses from high and low silica treatments were measured using methods detailed above ($n$ =10) (Table 2). To confirm that our manipulated silica levels were within the range occurring in natural communities, leaf samples of each of the five grass species were collected from 10 replicate plants in 10 sites around East Sussex in August 2005, selected to represent a range of soil types and habitats (Table 2).

Voles were limited to water and a dried diet for 24 hours prior to the trials. We conducted intraspecific paired feeding choice tests for all grass species between low and high silica treatments using three individual plants per treatment per trial. The leaf area of each plant was scanned at the start of the trials (AM-200 leaf area meter, ADC) and the plants arranged randomly in a grid design

(3×2 individuals) within a glass tank (45×30×30 cm) containing sawdust in the bottom, level with the base of the shoots of the grasses. One vole was then placed in the cage and left to feed. The stopping rule for trials was when an estimated 50% of total leaf area had been consumed, which took 1–2 hours for the trials with six plants, after which the remaining leaf area of each plant was measured. The interspecific multiple-choice preference trials on either high or low silica grasses of all five species were conducted in the same manner, with three individuals of each grass species per trial. Ten replicate trials were completed for each species and silica treatment.

## STATISTICAL ANALYSES

All correlation and regression analyses were carried out on species means as our hypotheses relate to between species differences. Due to the limitations on plant material, different plant quality measurements could not be performed on the same individual plants, but all plants were grown under identical conditions and analysed at the same age. To examine whether there was any connection between plant defence characteristics and both plant growth rate and nitrogen concentration, we used a series of Pearson's correlations. Pearson's correlations were also used to assess the relationship between the CDS of grass species (see above) and growth rates and nitrogen concentration of grass species.

To assess whether voles displayed significant feeding preferences between the 18 grass species, we calculated the amount of leaf area eaten for each species as a proportion of the total leaf area consumed in the trial. This accounted for the large degree of interspecific variation in plant size. Data were arcsine square-root transformed before carrying out a one-way ANOVA with trial as replicate. Forward step-wise regression analysis was used to assess which plant based factors influenced the amount of leaf area of each species consumed by voles. Following methodology by Hartley *et al.* (1997), the criterion for inclusion in the final regression model was that a factor must

add 4.0 to the variance ratio. This analysis was used to assess a) which factors had the greatest influence on vole feeding preference across all factors measured; b) which of the four defensive factors (silica, and phenolic concentrations, toughness and abrasiveness) had the greatest influence on vole feeding preference; c) which factors had the greatest influence on vole feeding preference when considering defences as the CDS of each grass species and all other factors (excluding toughness, silica and phenolic concentrations individually).

Linear regression was used to assess the relationship between silica concentration and abrasiveness for a) all 18 species sampled and b) the change in silica concentration and change in abrasiveness for the five species of which silica concentration was manipulated i.e. *A. capillaris*, *B. pinnatum*, *F. ovina*, *L. perenne* and *P. annua*.

For the intraspecific feeding trials with silica manipulated plants, the leaf area consumed from all three plants per silica treatment was pooled to calculate the total leaf area consumed per damage treatment per trial. Feeding preferences were then compared using paired t-tests. For the interspecific preferences on silica manipulated plants, the leaf area consumed from all three plants per species was pooled to calculate the total leaf area consumed per species per trial. Feeding preferences were then compared using mixed-model two-way ANOVA's, with species and trial as factors and trial as a random factor. We then carried out Tukey's *post-hoc* analysis of significant results.

## Results

### THE RAH AND GROWTH DEFENCE ALLOCATIONS ACROSS GRASS SPECIES

We predicted that defence allocation would be negatively correlated with species growth rate and foliar nitrogen concentration. However, we found no significant correlations between grass growth

rate and the levels of any individual defence (Fig. 1). In contrast, there were clear negative correlations between silica concentration and foliar nitrogen concentration (Fig. 1b), and between abrasiveness and foliar nitrogen concentration (Fig. 1d); but not between foliar nitrogen concentration and either leaf toughness or phenolic concentration (Fig. 1f & h).

The grass species exhibited defence strategies that focused on different types of defence. For example, *A. elatius* had very tough leaves (43% of its CDS), while investing very little in silica (19%), while *D. caespitosa* had only moderately toughened leaves (21%) but very high silica concentration (51%) (Fig. 2). The CDS differed dramatically between species, as did the relative investment in each defence type assessed, i.e. phenolic concentration, silica concentration and leaf toughness (Fig. 2). When assessing the CDS across 18 species, we found strong negative correlations between this parameter and both plant growth rate (Fig. 3a) and foliar nitrogen concentration (Fig. 3b).

PLANT BASED FACTORS AND VOLE FEEDING PREFERENCES ACROSS ALL SPECIES

Voles displayed a high level of selectivity in feeding preference between different grass species (Fig. 4a), with strongest preference for *P. annua* and *L. perenne*. Most of the plant characteristics did not have a significant influence on vole feeding preferences and therefore, were not included in the stepwise regression model. The most influential factor on vole feeding choice was the growth rate of the grass species, followed by the total nitrogen concentration (Table 3a). Together these factors explained 55% of the variation in feeding choice by voles. The first defensive factor significantly influencing vole feeding choice, when all plant characteristics were included in the model, was phenolic concentration. This explained an additional 15% of the variation, despite the very low levels of phenolics in all grass species studied. However, when assessing the influence on vole feeding preference of each defensive factor in isolation, it was silica that had the greatest

impact ($r^2$ = 35.8%, Table 3b), followed by phenolic concentration ($r^2$ = 21.4%, Table 3b). There was a very strong relationship between silica and nitrogen concentration which appears to be the primary reason that silica did not have a greater influence in the first analysis (Table 3a). When assessing vole feeding preference in relation to CDS and all other parameters measured, the most influential factor was the defence investment ($r^2$ = 55.1%, Fig. 4b, Table 3c), with an additional 10% of the variation explained by both plant growth rate and specific leaf area.

## MANIPULATION OF SILICA CONCENTRATION AND ABRASIVENESS AND VOLE FEEDING PREFERENCE

The silica concentrations of the 18 species significantly affected leaf abrasiveness: 70.2% of the variation in abrasiveness across species was explained by the foliar silica concentration alone (Fig. 5a). For five of the 18 species we manipulated silica concentrations and measured the effect on abrasiveness. The change in silica concentration within species explained 78.3% of the variation in the subsequent change in abrasiveness for the same species (Fig. 5b).

Higher concentrations of silica in the leaves of grasses deterred feeding by voles on three of the five grass species tested (Fig. 6a), although there was large variation in the amount of leaf area consumed between species. For each of the species for which higher silica concentration deterred feeding by voles, the silica addition treatment significantly affected both the silica concentration of leaves and their abrasiveness (Table 2). For two of the species tested, silica did not affect vole feeding preference. *P. annua* was extremely palatable on both treatments with high concentration of nitrogen in leaves, low phenolic concentration and low uptake of silica which was not sufficient to significantly affect abrasiveness (Table 2). Although in *A. capillaris* the silica treatment did increase both the silica content and the abrasiveness of leaves, it had low palatability in both silica treatments, with relatively high phenolic concentration and low nitrogen concentration.

The silica addition treatment also affected the relative palatability of the five species. In the absence of silica, *L. perenne* was significantly preferred over each of the other species tested (Fig. 6b). However, for plants from the silica addition treatment a clear preference rank was detected, with *A. capillaris* and *B. pinnatum* displaying extremely low palatability, followed by *F. ovina*, and with clear separation of preference between *P. annua* and *L. perenne* (Fig. 6c). Preference ranks found in the absence of silica matched approximately those that would be predicted by nitrogen concentration and the relative growth rates of the grasses (Table 2). In contrast, in the silica addition treatment the preference rank no longer matches nitrogen concentrations, but instead reflects the interspecific variation in leaf silica concentration (Table 2).

## Discussion

### THE RAH AND DEFENCE ALLOCATIONS ACROSS GRASS SPECIES

We found strong evidence in support of the RAH when assessing the CDS of grass species. Defence levels were negatively correlated with both plant growth rate and foliar nitrogen concentration. Similar patterns of defence strategies have been demonstrated for woody plant species. For example, Coley (1988) found that the growth rates of 41 tropical tree species were negatively correlated with an estimate of overall defence investment derived from the relative effectiveness of ten defensive characteristics. However, this is the first study to demonstrate a similar result in grasses.

When we considered the investment of all 18 species in each defence individually, and compared them with plant growth rates, we found a variable picture with respect to the RAH because different grass species allocated differentially in different types of defence, thus highlighting the need for a measure of overall defence strategy in interspecific comparisons. For example, *B. media* and *D.*

*caespitosa* have similar defence investment ratings; however, *D. caespitosa* invests highly in silica-based defences, while *B. media* has low silica concentrations but high phenolics concentrations in leaf tissue. Our findings were consistent with previous studies looking at single defensive factors and plant growth rates. Hjältén *et al.* (1996) compared 20 plant species (grasses, forbs and shrubs) for defence investment, growth strategies and palatability to voles, and concluded that more information is required on the overall causes of reduced palatability in plants to make adequate predictions based on growth strategies. In our study, the grass species differed markedly in their allocation to defence as well as growth rate. These two factors may affect their ability to re-grow after herbivory, given the trade-offs between compensation and defence which have been shown in many systems (Leimu & Koricheva 2006).

The differential investment in different defence types between species may reflect both the environmental adaptations of each of the species and the principle type of herbivory to which the species are exposed. For example, silica is taken up from the soil as monosilicic acid, the availability of which is dependant on soil type and pH (O'Reagain & Mentis 1989). Therefore, in environments of high pH and low silica availability in the soil, silica defences may play only a minor role in defence. Also, not all defences are effective to all herbivores. Discrete phytoliths of silica within the leaves are not an effective defence against phloem feeding insects (Massey *et al.* 2006), which appear to be able to avoid them, whereas folivores such as voles are unable to do so.

Defences are also likely to differ in the cost to the plant. Although silica is an effective mechanism for deterring vole feeding, not all species provision leaves with the same concentration of silica. Unlike many carbon- or nitrogen-based antiherbivore defences, where resources involved can be allocated either to growth or defence (Bryant *et al.* 1983), silica is not directly associated with plant growth (Raven 2003). However, recent work has shown that silica is actively transported into roots

in silicon-accumulating plants (Ma *et al.* 2006), implying a cost associated with uptake. We found that for the five species grown at high and low silica concentrations, the mean growth rate tended to be lower in the plants which had taken up more silica (Table 2a). Although none of these differences were found to be significant over this short time period, they do suggest that there may be a cost to silica uptake in the longer term. Further studies are required to determine the actual cost of silica uptake for plants.

PLANT-BASED FACTORS AND VOLE FEEDING PREFERENCES ACROSS ALL SPECIES

As with previous studies, plant growth rates and nitrogen concentrations were the most important single predictors of vole feeding preferences (Marquis & Batzli 1989; Hartley *et al.* 1995; Hjältén *et al.* 1996), although this is the first study to demonstrate this for the primary food source; i.e. grasses, of *Microtus* voles (Ostfeld 1985). Previous studies have assessed vole feeding preference using a diverse range of plant species and consistently found that voles select plants high in foliar nitrogen concentration, with defence levels being of secondary importance (Marquis & Batzli 1989; Hjältén *et al.* 1996). This was also the case in our study. The growth rates and foliar nitrogen concentration of grasses explained 55% of the variation in vole feeding choice, while significant defences explained only 29%. However, when considering only defence characteristics, the silica concentration of grasses was the best predictor of vole feeding preference, explaining almost as much variation as growth rate ($r^2 = 36\%$ *vs*. $r^2 = 40\%$ respectively). This finding supports other recent evidence that silica is the primary defence against folivores in many grasses (Gali-Muhtsaib *et al.* 1992; Massey *et al.* 2006; Massey & Hartley 2006), and thus is important for our understanding the relative palatability of grasses to herbivores.

In addition to considering single defence factors, we also analysed the effect of all defences together as the CDS on vole feeding preferences. In this case, the CDS was a stronger predictor of vole

feeding preference than nitrogen concentration or growth rate. This suggests that it is a combination of defences that affect vole feeding, rather than a single type of defence. This may be particularly important to generalist feeding herbivores, such as voles, that can feed on a wide range of species and encounter a broad spectrum of defences.

## EFFECTS OF SILICA CONCENTRATION AND ABRASIVENESS MANIPULATION ON VOLE FEEDING PREFERENCE

We have demonstrated that, both within species and across a range of species, higher silica concentration increases the abrasiveness of leaf tissues. By manipulating the concentration of silica in five grass species we found that the increase in abrasiveness was correlated with the increase in foliar silica concentration. As there were no changes in nitrogen concentration, phenolic concentration or growth rates associated with the silica manipulation, we have shown that the increase in abrasiveness due to silica is the probable mechanism for feeding deterrence. For three of the five species tested, an increase in silica concentration deterred feeding by voles. In addition, we have shown that silica has the potential to alter the feeding preference ranks between species, due to interspecific variation in silica uptake. The foliar silica concentrations obtained in this study were within the ranges of natural variation observed in field samples (Table 2). Therefore, silica may also be an important determinant of the relative feeding preference ranks between environments due to spatial variation in silica concentrations caused by different biotic and environmental variables, including grazing history (McNaughton & Tarrants 1983; Massey & Hartley 2006), soil pH and substrate type (O'Reagain & Mentis 1989). Interspecific and intraspecific variation in silica concentration between environments, as well as the highly selective nature of vole feeding, could ultimately result in changes in plant species abundance and, hence, the composition of plant communities (Moen *et al.* 1993; Howe & Brown 1999; Clay 2001; Howe & Lane 2004).

## CONCLUSIONS

Defence allocation strategies have often featured as a minor component in the study of grass palatability (Frazer & Grime 1999; Del-Val & Crawely 2004), possibly due to the extent to which many grasses have adapted to compensate for biomass lost to herbivory. However, this study highlights the importance of defence to grass-herbivore interactions. Both the CDS in relation to species growth rate, as well as vole feeding preference in relation to CDS, support predictions of the RAH (Coley *et al.* 1985). Specifically, grasses with faster growth rates had lower levels of defences and were more palatable to voles than slower-growing grass species. In addition, our study has highlighted the role of silica-based defences in determining the palatability of grasses to generalist grass-feeding herbivores, such as voles.

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**References**

Allen, S.E. (1989) *Chemical Analysis of Ecological Materials*. Second Edition. Blackwell Press, London, UK.

Bryant, J.P., Chapin, F.S., & Klein, D.R. (1983) Carbon nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos*, **40**, 357-368.

Choong, M.F. (1996) What makes a leaf tough and how this affects the pattern of *Castanopsis fissa* leaf consumption by caterpillars. *Functional Ecology*, **10**, 668-674.

Clay, K. (2001) Symbiosis and the regulation of communities. *American Zoologist*, **41**, 810-824.

Coley, P.D. (1980) Effects of leaf age and plant life-history patterns on herbivory. *Nature*, **284**, 545-546.

Coley, P.D. (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological Monographs*, **53**, 209-233.

Coley, P.D. (1988) Effects of plant-growth rate and leaf lifetime on the amount and type of anti-herbivore defense. *Oecologia*, **74**, 531-536.

Coley, P.D., Bryant, J.P., & Chapin, F.S. (1985) Resource availability and plant antiherbivore defense. *Science*, **230**, 895-899.

Del-Val, E.K. & Crawley, M.J. (2004) Importance of tolerance to herbivory for plant survival in a British grassland. *Journal of Vegetation Science*, **15**, 357-364.

Del-Val, E.K. & Crawley, M.J. (2005) Are grazing increaser species better tolerators than decreasers? An experimental assessment of defoliation tolerance in eight British grassland species. *Journal of Ecology*, **93**, 1005-1016.

Gali-Muhtasib, H.U., Smith, C.C., & Higgins, J.J. (1992) The effect of silica in grasses on the feeding-behavior of the prairie vole, *Microtus-ochrogaster*. *Ecology*, **73**, 1724-1729.

Grime, J.P. (1979) *Plant Strategies and Vegetation Processes*. John Wiley and Sons, New York, USA.

Grime, J.P., Hodgson, J.G. & Hunt, R. (1988) *Comparative Plant Ecology: a Functional Approach to Common British Species*. Unwin Hyman, London, UK.

Grime, J.P., Cooper, J.E.L., & Tasker, D. (1993) Tearability. *Methods in Comparative Plant Ecology: a Laboratory Manual* (eds G.A.F. Herndry & J.P. Grime), pp. 121-123. Chapman & Hall, London, UK.

Hartley, S.E. & Jones, C.G. (1993) Plant chemistry and herbivory, or why the world is green. *Plant Ecology* (ed M.J. Crawley), pp. 284-324. Blackwell Press, London, UK.

Hartley, S.E., Nelson, K., & Gorman, M. (1995) The effect of fertilizer and shading on plant-chemical composition and palatability to Orkney voles, *Microtus-arvalis orcadensis*. *Oikos*, **72**, 79-87.

Hartley, S.E., Iason, G.R., Duncan, A.J., & Hitchcock, D. (1997) Feeding behaviour of Red Deer (*Cervus elaphus*) offered Sitka Spruce saplings (*Picea sitchensis*) grown under different light and nutrient regimes. *Functional Ecology*, **11**, 348-357.

Herms, D.A. & Mattson, W.J. (1992) The dilemma of plants - to grow or defend. *Quarterly Review of Biology*, **67**, 283-335.

Hjältén, J., Danell, K., & Ericson, L. (1996) Food selection by two vole species in relation to plant growth strategies and plant chemistry. *Oikos*, **76**, 181-190.

Howe, H.F. & Brown, J.S. (1999) Effects of birds and rodents on synthetic tallgrass communities. *Ecology*, **80**, 1776-1781.

Howe, H.F. & Lane, D. (2004) Vole-driven succession in experimental wet-prairie restorations. *Ecological Applications*, **14**, 1295-1305.

Leimu, R. & Koricheva, J. (2006) A meta-analysis of tradeoffs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. *Oikos*, **112**, 1-9.

Marquis, R.J. & Batzli, G.O. (1989) Influence of chemical factors on palatability of forage to voles. *Journal of Mammalogy*, **70**, 503-511.

Ma, J.F., Tamai, K., Yamaji, N., Mitani, N., Konishi, S., Katsuhara, M., Ishiguro, M., Murata, Y. & Yano, M. (2006) A silicon transporter in rice. *Nature,* **440,** 688-691.

Massey, F.P., Ennos, A.R., & Hartley, S.E. (2006) Silica in grasses as a defence against insect herbivores: contrasting effects on folivores and a phloem feeder. *Journal of Animal Ecology*, **75**, 595-603.

Massey, F.P., Press, M.C., & Hartley, S.E. (2005) Long- and short-term induction of defences in seedlings of *Shorea leprosula* (Dipterocarpaceae): support for the carbon: nutrient balance hypothesis. *Journal of Tropical Ecology*, **21**, 195-201.

Massey, F.P. & Hartley, S.E. (2006) Experimental demonstration of the antiherbivore effects of silica in grasses: impacts on foliage digestibility and vole growth rates. *Proceedings of the Royal Society of London , Series B – Biological Sciences*, **273**, 2299-2304.

Matsuki, S. & Koike, T. (2006) Comparison of leaf life span, photosynthesis and defensive traits across seven species of deciduous broad-leaf tree seedlings. *Annals of Botany*, **97**, 813-817.

McNaughton, S.J. (1983) Compensatory plant-growth as a response to herbivory. *Oikos*, **40**, 329-336.

McNaughton, S.J. (1992) Laboratory-simulated grazing - interactive effects of defoliation and canopy closure on Serengeti grasses. *Ecology*, **73**, 170-182.

McNaughton, S.J. & Tarrants, J.L. (1983) Grass leaf silicification - natural-selection for an inducible defense against herbivores. *Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences*, **80**, 790-791.

Moen, J., Gardfjell, H., Oksanen, L., Ericson, L., & Ekerholm, P. (1993) Grazing by food-limited microtine rodents on a productive experimental plant community - does the green desert exist. *Oikos*, **68**, 401-413.

O'Reagain, P.J. & Mentis, M.T. (1989) Leaf silification in grasses- A review. *Journal of the Grassland Society of South Africa*, **6**, 37-43.

Ostfeld, R.S. (1985) Limiting resources and territoriality in microtine rodents. *American Naturalist*, **126**, 1-15.

Raven, J.A. (2003) Cycling silicon - the role of accumulation in plants - Commentary. *New Phytologist*, **158**, 419-421.

1 Stamp, N. (2003) Out of the quagmire of plant defense hypotheses. *Quarterly Review of Biology*,
2 **78**, 23-55.
3 Vicari, M. & Bazely, D.R. (1993) Do grasses fight back - the case for antiherbivore defenses.
4 *Trends in Ecology & Evolution*, **8**, 137-141.

1 Table 1: Comparison of growth and defence characteristics of 18 study species, including species codes used in figures. Growth conditions are

2 detailed in the methods.

| Species | Species code | Growth rate (mg $DM.d^{-1}$) | Nitrogen concentration (% DM) | Silica concentration (% DM) | Abrasiveness ($R_z$ in μm) | Toughness ($g.cm^{-2}$) | Phenolic concentration (% DM) |
|---|---|---|---|---|---|---|---|
| *Agrostis capillaris* | AC | 45.20 | 2.29 | 2.17 | 3.12 | 114.09 | 1.91 |
| *Arrhenatherum elatius* | AE | 21.19 | 2.57 | 1.98 | 3.15 | 148.56 | 1.75 |
| *Anthoxanthum odoratum* | AO | 8.30 | 3.36 | 2.21 | 3.45 | 66.87 | 2.22 |
| *Agrostis vinealis* | AV | 5.14 | 1.78 | 3.03 | 2.96 | 118.44 | 1.73 |
| *Bromopsis erecta* | BE | 13.64 | 1.80 | 3.05 | 3.62 | 118.12 | 2.20 |
| *Briza media* | BM | 9.54 | 2.89 | 2.69 | 3.41 | 66.48 | 3.30 |
| *Brachypodium pinnatum* | BP | 6.69 | 2.14 | 4.10 | 4.31 | 189.54 | 1.59 |
| *Calamagrostis epigejos* | CE | 6.28 | 1.94 | 3.85 | 3.46 | 152.30 | 1.48 |
| *Dactylis glomerata* | DG | 53.72 | 2.01 | 2.98 | 2.84 | 167.25 | 2.32 |
| *Danthonia decumbens* | DD | 9.87 | 1.43 | 3.26 | 3.00 | 100.25 | 2.59 |
| *Deschampsia caespitosa* | DC | 32.50 | 1.51 | 6.25 | 4.68 | 88.24 | 1.59 |
| *Deschampsia flexuosa* | DF | 12.90 | 1.42 | 4.11 | 3.95 | 131.60 | 1.12 |
| *Festuca ovina* | FO | 10.46 | 1.65 | 3.85 | 4.24 | 72.38 | 1.24 |
| *Festuca rubra* | FR | 10.23 | 3.27 | 3.20 | 3.09 | 140.70 | 1.89 |
| *Holcus lanatus* | HL | 25.20 | 1.93 | 2.69 | 3.28 | 83.30 | 1.35 |
| *Lolium perenne* | LP | 77.66 | 2.59 | 2.15 | 3.32 | 139.60 | 1.14 |
| *Nardus stricta* | NS | 0.96 | 1.12 | 6.93 | 4.59 | 236.50 | 2.34 |
| *Poa annua* | PA | 72.40 | 3.57 | 1.23 | 2.38 | 42.56 | 1.45 |

Table 2: a) Growth rate, leaf characteristics and chemical composition of five grass species under high and low silica treatments ([†] ns = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). b) The foliar silica concentration of study species from field sites (minimum–maximum values in plants collected from 10 different natural plant communities).

| a) | Silica treatment | Growth rate (mg DM.d$^{-1}$) | Leaf nitrogen concentration (% DM) | Silica concentration (% DM) | Phenolic concentration (% DM) | Abrasiveness ($R_z$ in µm) | b) Field silica concentration (% DM) |
|---|---|---|---|---|---|---|---|
| *Agrostis capillaris* | Low | 14.8 ± 1.7 | 2.29 ± 0.26 | 0.46 ± 0.03 | 1.90 ± 0.09 | 2.56 ± 0.15 | 0.75 – 2.56 |
| | High | 14.1 ± 1.4 | 2.27 ± 0.18 | 2.51 ± 0.14 | 1.92 ± 0.07 | 3.38 ± 0.16 | |
| *Brachypodium pinnatum* | Low | 2.5 ± 0.6 | 1.44 ± 0.10 | 0.47 ± 0.03 | 1.20 ± 0.10 | 2.57 ± 0.11 | 0.89 – 5.47 |
| | High | 2.1 ± 0.4 | 1.56 ± 0.15 | 2.87 ± 0.14 | 1.17 ± 0.10 | 3.92 ± 0.15 | |
| *Festuca ovina* | Low | 4.8 ± 1.2 | 2.11 ± 0.18 | 0.52 ± 0.04 | 1.27 ± 0.05 | 3.14 ± 0.12 | 0.65 – 3.54 |
| | High | 4.3 ± 0.7 | 2.16 ± 0.19 | 2.44 ± 0.16 | 1.35 ± 0.05 | 4.03 ± 0.13 | |
| *Lolium perenne* | Low | 38.8 ± 4.0 | 3.24 ± 0.10 | 0.54 ± 0.10 | 1.38 ± 0.05 | 2.63 ± 0.32 | 0.58 – 4.52 |
| | High | 33.8 ± 5.9 | 3.22 ± 0.09 | 4.68 ± 0.34 | 1.38 ± 0.05 | 4.15 ± 0.41 | |
| *Poa annua* | Low | 37.3 ± 5.4 | 3.53 ± 0.22 | 0.81 ± 0.15 | 1.08 ± 0.06 | 2.55 ± 0.13 | 0.27 – 2.97 |
| | High | 28.7 ± 5.2 | 3.33 ± 0.15 | 1.91 ± 0.22 | 1.22 ± 0.08 | 2.91 ± 0.12 | |
| ANOVA[†] | Species (S) | *** | *** | *** | *** | *** | |
| | Treatment (T) | ns | ns | *** | ns | *** | |
| | S × T | ns | ns | *** | ns | ns | |

1 Table 3: Summary of forward stepwise regression analysis for vole feeding preferences over 18
2 grass species. Numbers in parentheses indicate number of predictive factors considered in model.
3

| Variable | SE | *F* | df | *P* | Cumulative $r^2$(%) |
|---|---|---|---|---|---|
| (a) All factors (12) | | | | | |
| Growth rate | 0.031 | 12.395 | 1 | 0.003 | 40.1 |
| Nitrogen | 0.027 | 6.352 | 1 | 0.024 | 55.1 |
| Phenolic concentration | 0.022 | 8.469 | 1 | 0.011 | 70.0 |
| C:N ratio | 0.020 | 4.194 | 1 | 0.090 | 74.4 |
| Silica concentration | 0.018 | 4.347 | 1 | 0.063 | 79.4 |
| Abrasiveness | 0.013 | 11.211 | 1 | 0.006 | 88.9 |
| (b) Defensive factors (4) | | | | | |
| Silica concentration | 0.033 | 8.918 | 1 | 0.009 | 35.8 |
| Phenolic concentration | 0.028 | 7.502 | 1 | 0.015 | 57.2 |
| (c) Cumulative defence score and other factors (9) | | | | | |
| Defence score | 0.027 | 21.860 | 1 | <0.001 | 55.1 |
| Growth rate | 0.024 | 5.753 | 1 | 0.030 | 65.4 |
| SLA | 0.020 | 6.855 | 1 | 0.020 | 75.1 |

**Figure legends**

**Fig. 1**: Relationship between growth rates, foliar nitrogen concentration and: (**a,b**) silica concentration; (**c**,**d**) abrasiveness; (**e**,**f**) toughness; (**g**,**h**) phenolic concentration of 18 grass species. Correlation coefficients and *P*-values for Pearson's correlations are shown.

**Fig. 2**: Cumulative defence score for each of 18 grass species (see methods for details) as relative investment in each defence type measured: silica concentration, phenolic concentration and leaf toughness. Species codes: AC: *Agrostis capillaris*, AV: *Agrostis vinealis*, AO: *Anthoxanthum odoratum*, AE: *Arrhenatherum elatius*, BP: *Brachypodium pinnatum*, BM: *Briza media*, BE: *Bromopsis erecta*, CE: *Calamagrostis epigejos*, DG: *Dactylis glomerata*, DD: *Danthonia decumbens*, DC: *Deschampsia caespitosa*, DF: *Deschampsia flexuosa*, FO: *Festuca ovina*, FR: *Festuca rubra*, HL: *Holcus lanatus*, LP: *Lolium perenne*, NS: *Nardus stricta*, PA: *Poa annua*.

**Fig. 3**: Relationship between cumulative defence score (see methods for details) and (**a**) plant growth rates and (**b**) foliar nitrogen concentration of 18 grass species. Correlation coefficients and p-values for Pearson's correlations are shown.

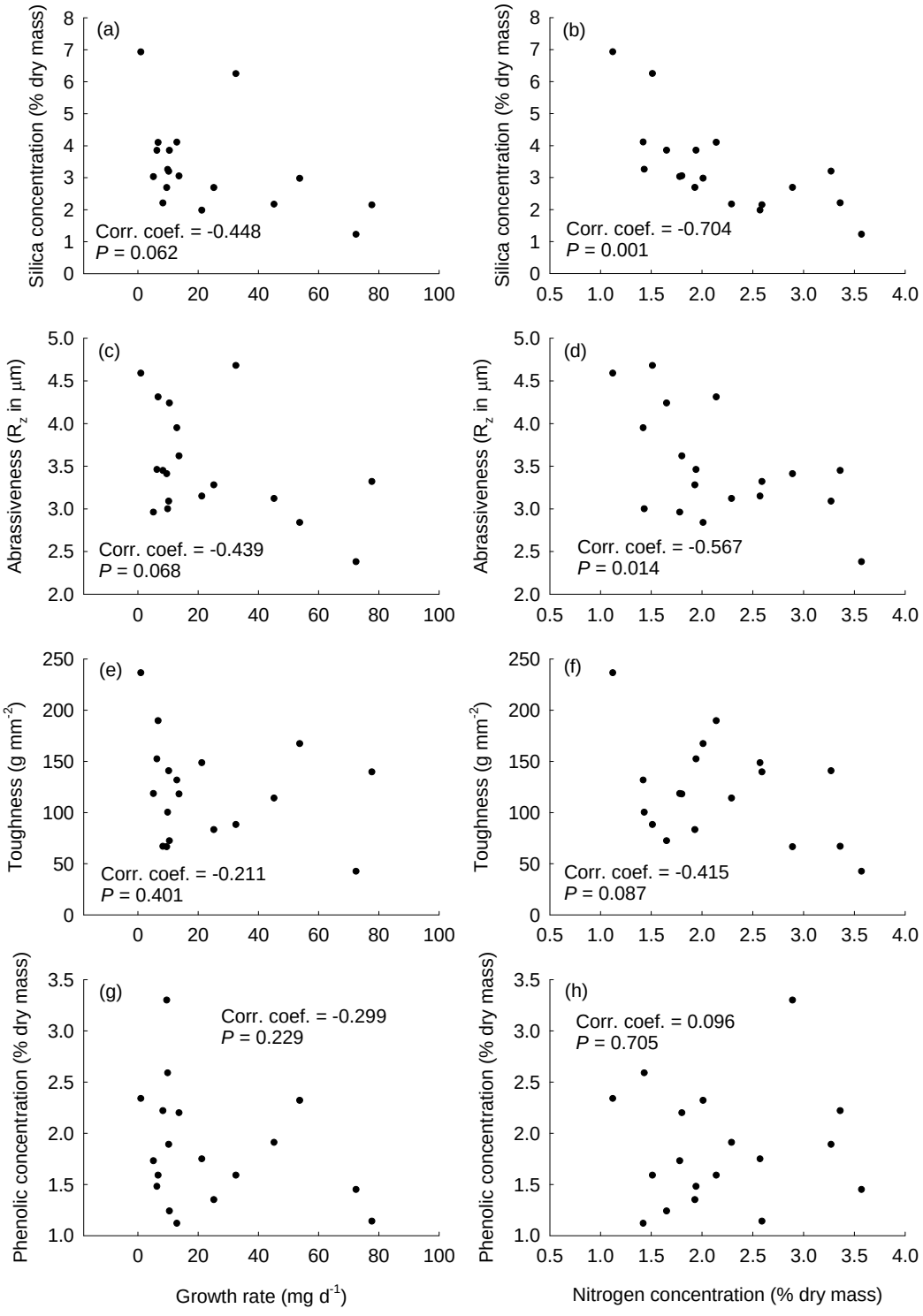
**Fig. 4**: (**a**) Relative feeding preferences of voles for 18 grass species. Values are trial means (±SE), ANOVA: $F_{17,162} = 12.84$, $P < 0.001$. (**b**) Relationship between cumulative defence score (see methods for details) and vole feeding preferences across 18 grass species. The line is described by the equation y = 0.000766x + 0.174 ($F_{1,16} = 21.86$, $P < 0.001$; $r^2 = 55.1$, $P < 0.001$). Species codes: AC: *Agrostis capillaris*, AV: *Agrostis vinealis*, AO: *Anthoxanthum odoratum*, AE: *Arrhenatherum elatius*, BP: *Brachypodium pinnatum*, BM: *Briza media*, BE: *Bromopsis erecta*, CE: *Calamagrostis epigejos*, DG: *Dactylis glomerata*, DD: *Danthonia decumbens*, DC: *Deschampsia caespitosa*, DF:

*Deschampsia flexuosa*, FO: *Festuca ovina*, FR: *Festuca rubra*, HL: *Holcus lanatus*, LP: *Lolium perenne*, NS: *Nardus stricta*, PA: *Poa annua*.

**Fig. 5**: (**a**) Relationship between foliar silica concentration and abrasiveness for 18 grass species. The line is described by the equation y = 0.367x + 2.28 ($F_{1,16}$ = 37.66, $P < 0.001$; $r^2$ = 70.2, $P < 0.001$). (**b**) Relationship between change in silica concentration and change in abrasiveness due to the silica addition treatment for five grass species (*Agrostis capillaris*, *Brachypodium pinnatum*, *Festuca ovina*, *Lolium perenne* and *Poa annua*). The line is described by the equation y = 0.392x + 0.092 ($F_{1,3}$ = 15.42, $P$ = 0.029; $r^2$ = 78.3, $P$ = 0.029). See Table 1 for species codes.

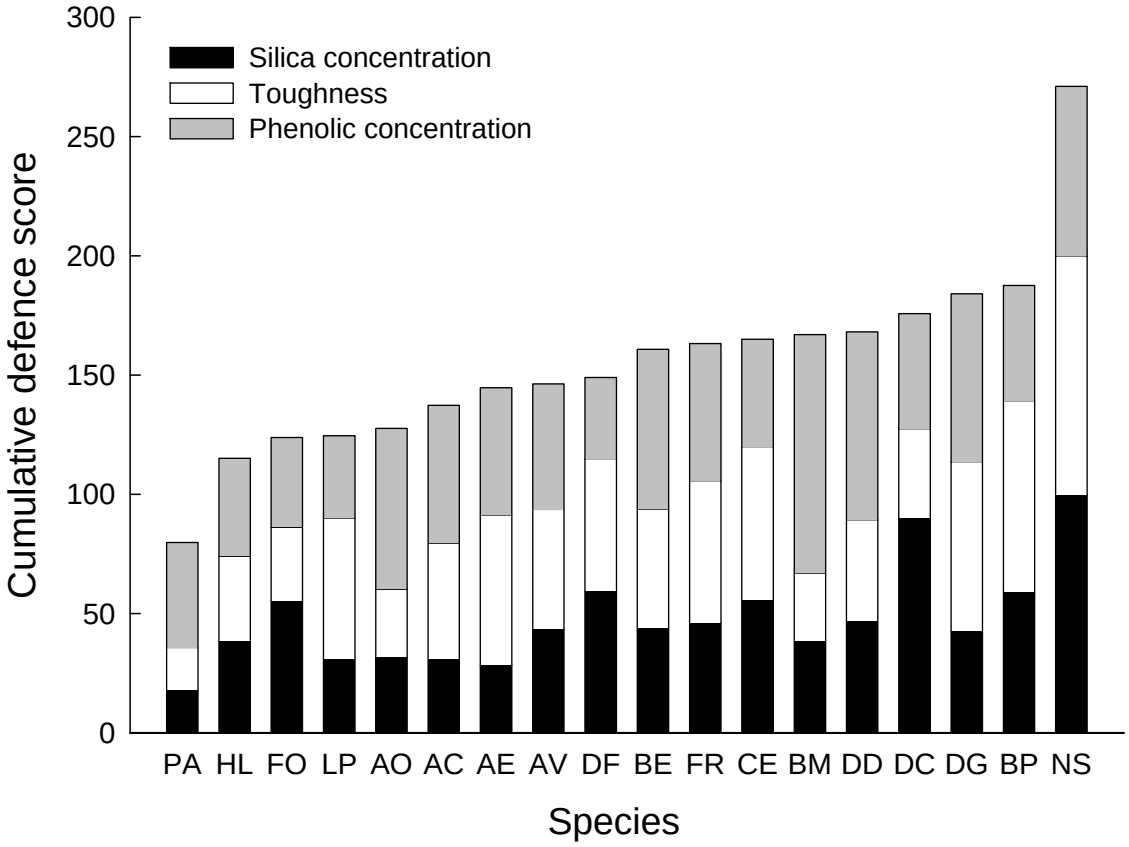
**Fig. 6**: (**a**) Intraspecific feeding preferences as leaf area eaten by voles for grasses grown in low silica (open bars) and high silica (closed bars) treatments. Values are means of trial means from pair-wise intraspecific preference tests (±SE). (Degrees of significance from paired t-tests using trial means for each species are indicated as follows: ns = not significant, *** $P < 0.001$). Interspecific feeding preferences as leaf area eaten by voles of grasses grown in(**b**) low silica and (**c**) high silica treatments (ANOVA low silica: trial $F_{9,36}$ = 0.41, $P$ = 0.924, treatment $F_{4,36}$ = 18.52, $P < 0.001$; high silica: trial $F_{9,36}$ = 2.87, $P$ = 0.012, treatment $F_{4,36}$ = 29.77, $P < 0.001$) . Bars within each graph not sharing a common letter differ significantly (Tukey's test $P < 0.05$). Species codes: AC = *Agrostis capillaris*, BP = *Brachypodium pinnatum*, FO = *Festuca ovina*, LP = *Lolium perenne*, PA = *Poa annua*.

1 Fig. 1


(a)
Silica concentration (% dry mass)
Corr. coef. = -0.448
P = 0.062
(b)
Silica concentration (% dry mass)
Corr. coef. = -0.704
P = 0.001
(c)
Abrassiveness (Rz in µm)
Corr. coef. = -0.439
P = 0.068
(d)
Abrassiveness (Rz in µm)
Corr. coef. = -0.567
P = 0.014
(e)
Toughness (g mm-2)
Corr. coef. = -0.211
P = 0.401
(f)
Toughness (g mm-2)
Corr. coef. = -0.415
P = 0.087
(g)
Phenolic concentration (% dry mass)
Corr. coef. = -0.299
P = 0.229
(h)
Phenolic concentration (% dry mass)
Corr. coef. = 0.096
P = 0.705
Growth rate (mg d-1)
Nitrogen concentration (% dry mass)

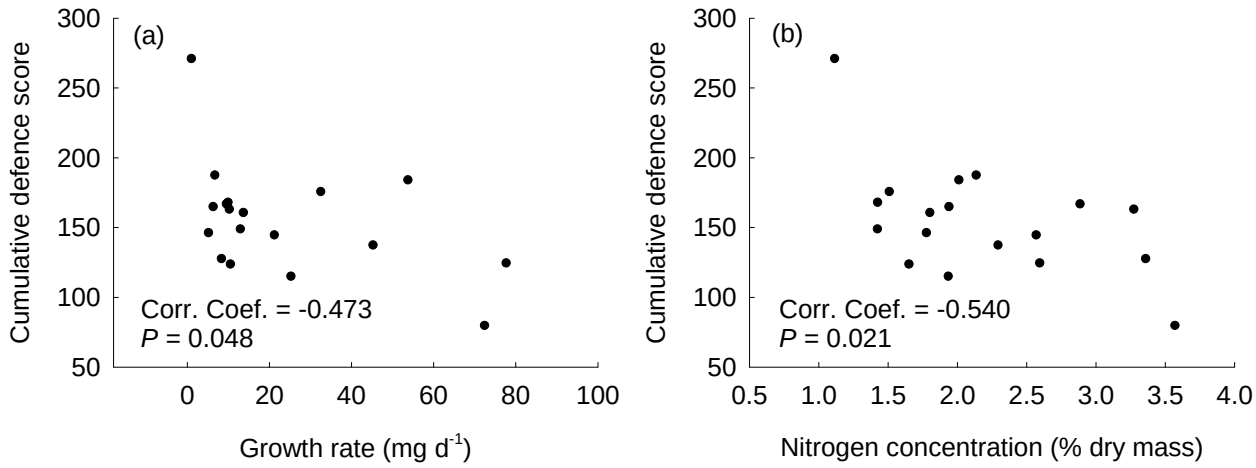
2

1 Fig. 2


Silica concentration
Toughness
Phenolic concentration
Cumulative defence score
300
250
200
150
100
50
0
PA HL FO LP AO AC AE AV DF BE FR CE BM DD DC DG BP NS
Species

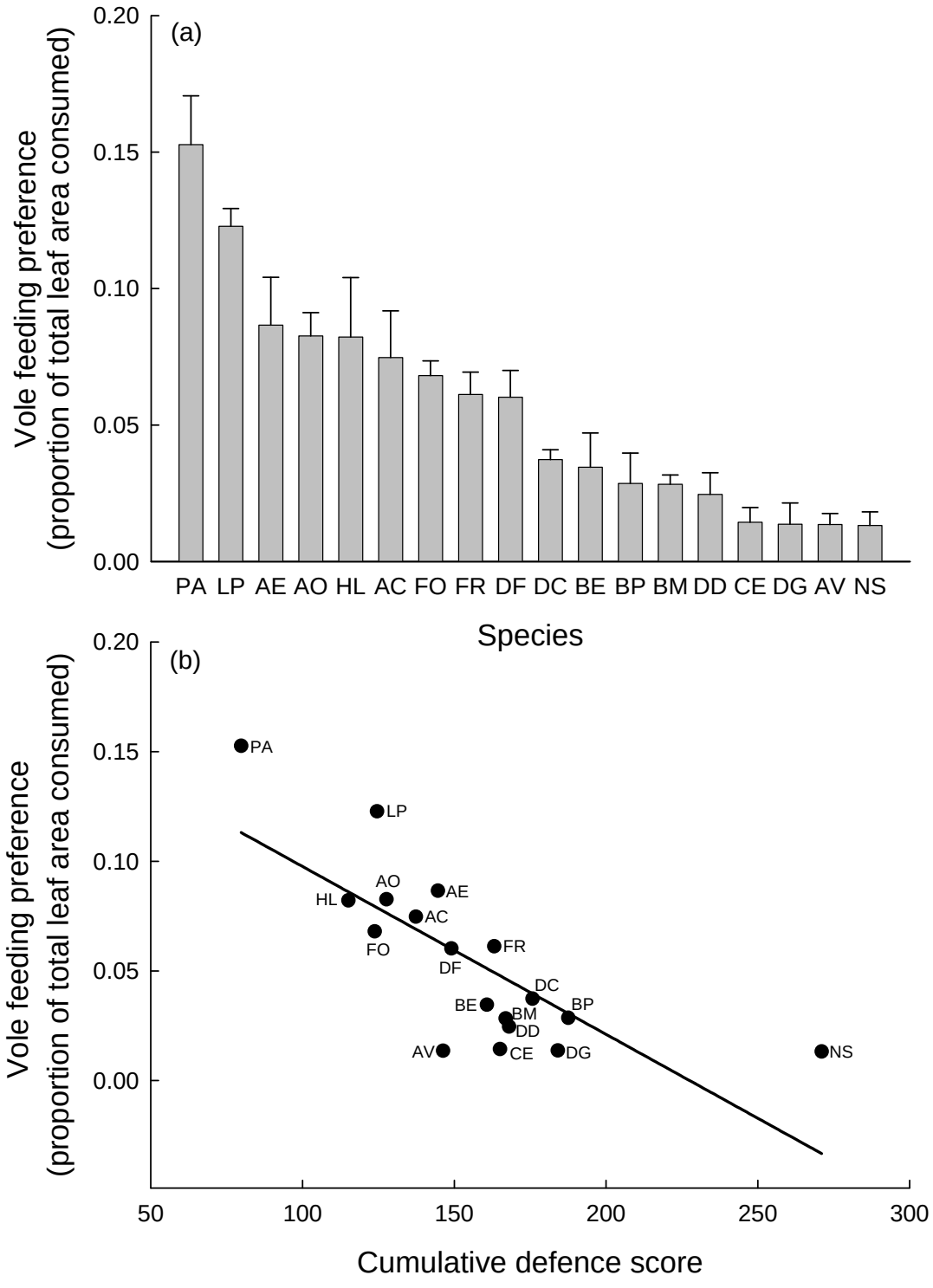
2

1 Fig. 3


(a)
Cumulative defence score
300
250
200
150
100
50
0
20
40
60
80
100
Growth rate (mg d-1)
Corr. Coef. = -0.473
P = 0.048
(b)
Cumulative defence score
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Nitrogen concentration (% dry mass)
Corr. Coef. = -0.540
P = 0.021

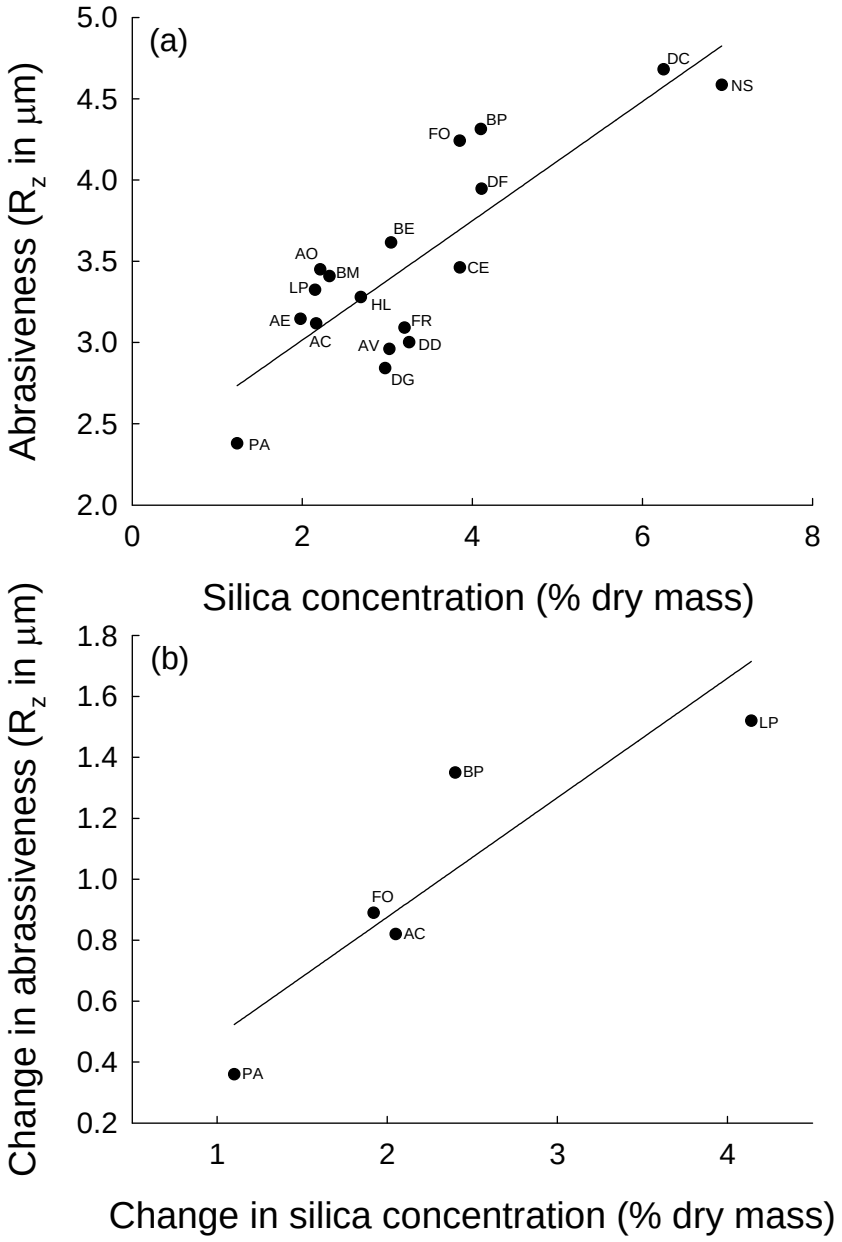
2

1 Fig. 4


(a)
Vole feeding preference
(proportion of total leaf area consumed)
0.20
0.15
0.10
0.05
0.00
PA LP AE AO HL AC FO FR DF DC BE BP BM DD CE DG AV NS
Species
(b)
Vole feeding preference
(proportion of total leaf area consumed)
PA
LP
AO
AE
HL
AC
FO
FR
DF
DC
BE
BP
BM
DD
AV
CE
DG
NS
50
100
150
200
250
300
Cumulative defence score

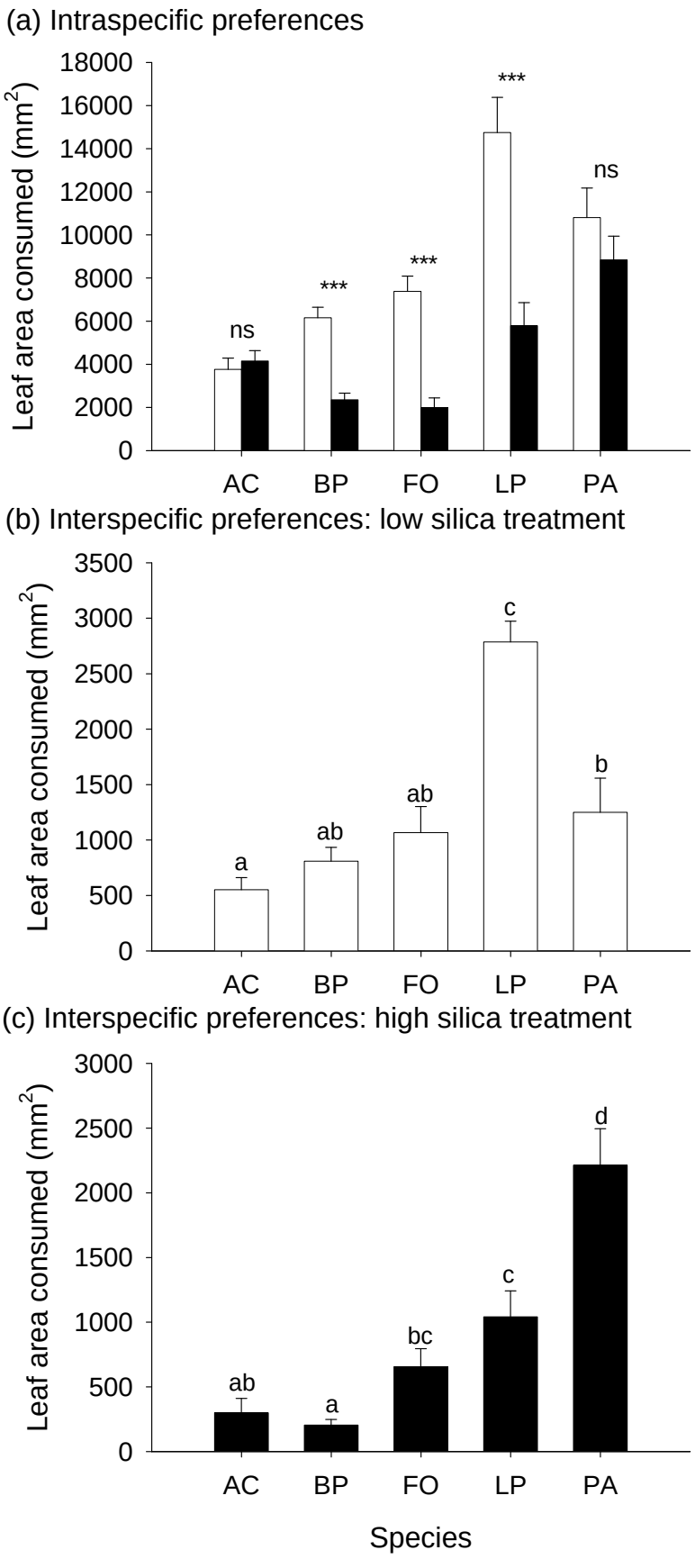
2

1 Fig. 5


(a)
Abrasiveness ($R_z$ in µm)
Silica concentration (% dry mass)
DC
NS
FO
BP
DF
BE
AO
BM
CE
LP
HL
AE
FR
AC
AV
DD
DG
PA
(b)
Change in abrassiveness ($R_z$ in µm)
Change in silica concentration (% dry mass)
LP
BP
FO
AC
PA

2

1 Fig 6


(a) Intraspecific preferences
Leaf area consumed (mm2)
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
ns
***
***
***
ns
AC
BP
FO
LP
PA
(b) Interspecific preferences: low silica treatment
Leaf area consumed (mm2)
3500
3000
2500
2000
1500
1000
500
0
a
ab
ab
c
b
AC
BP
FO
LP
PA
(c) Interspecific preferences: high silica treatment
Leaf area consumed (mm2)
3000
2500
2000
1500
1000
500
0
ab
a
bc
c
d
AC
BP
FO
LP
PA
Species

2